Comparative Properties of Hemolymph of the Gypsy Moth and the European Pine Sawfly

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Abstract: A number of properties of hemolymph were determined on two major forest pest insects: the gypsy moth and the European pine sawfly. The parameters measured include: pH, visible absorption spectra, sedimentation coefficients, electrophoretic patterns of proteins, lipids, carbohydrates, and isoenzymes, and amino acid analyses.

INTRODUCTION

The gypsy moth (*Lymantria dispar* Linnaeus), a lepidopteran, and the European pine sawfly (*Neodiprion sertifer* Geoffroy), a hymenopteran, are insect pests which annually cause extensive damage to trees. In the United States an active research program by the Department of Agriculture has been in progress on establishing suitable control measures for these insects.

Our knowledge of the biochemistry and physiology of the gypsy moth and the European pine sawfly is lacking, and in this regard the literature does not contain sufficient data on the properties of the hemolymph of either insect. The present study was concerned with resolving some of these deficiencies while providing a comparative analysis on the hemolymph of each insect.

MATERIALS AND METHODS

Insects. The gypsy moth and the European pine sawfly were obtained from local regions. Larvae of the gypsy moth were reared from egg masses on a synthetic diet (Vanderzant et al., 1962, ODell and Rollinson, 1966). Larvae of the European pine sawfly were collected from its hosts, Scotch pine and red pine. To offer a comparison of the properties of hemolymph on an approximate size basis, the 4th larval stage of the gypsy moth and the final larval stage of the European pine sawfly were used.

Hemolymph. Blood of either insect was obtained by pricking a proleg of a sufficient number of individuals and pooled without regard to sex. With time the blood of the gypsy moth melanizes when exposed to air. To inhibit the reaction a small crystal of phenylthiourea was added to gypsy moth blood in

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certain experiments. Blood of the European pine sawfly does not melanize when exposed to air.

pH. Values were obtained with a Corning Model 12 pH meter, using a microelectrode.

Visible Absorption Spectra. A Hitachi, Perkin-Elmer, Model 139 spectrophotometer was used. Blanks were standard phosphate buffers corresponding in pH to that of the blood of the insect measured.

Sedimentation Coefficient. Blood of the gypsy moth containing a small crystal of phenylthiourea or blood of the European pine sawfly were diluted with buffer (Miller and Golder, 1950). Sedimenting peaks were measured from Schlieren patterns obtained with a Model E analytical ultracentrifuge (Beckman Co.). The values obtained were corrected to standard conditions, $S_{20,w}$ (Schachman, 1959).

Polyacrylamide Gel Electrophoresis. Samples containing 10 μl of blood were electrophoresed anodically in 7.5% polyacrylamide gels (Davis, 1964), omitting the spacer gel (Lysenko, 1972). The system was run at 2.5 ma/tube (Wang and Patton, 1968) at 4°C in precooled (Hudson, 1966) buffer for 50 minutes. To allow for better current condition, the quantity of tris was increased in the running gel from 36.3 to 48.5 g, and in the sample gel from 5.98 to 9.6 g (Personal communication, E-C Apparatus Corp., 3831 Tyrone Blvd., N. Street, St. Petersburg, Florida). *Proteins* were stained in naphthalene black (lg/100 ml of 7% acetic acid) for 30 minutes, and destained with 7% acetic acid. Corresponding unstained gels were analyzed for lipoproteins, glycoproteins, and isoenzymes. *Lipoproteins* were stained for 24 hours in 100 ml of an aqueous solution containing 0.55 g sudan black, 7.5 ml acetic acid, and 70 ml ethanol. Destaining was accomplished through 2–3 changes of 7.5% acetic acid at room temperature. *Glycoproteins* were demonstrated by first fixing the gel samples in 7.5% acetic acid at room temperature for 1 hour, and then immersing the gels in 0.2% aqueous periodic acid, followed by refrigeration for 45 minutes. The gels were then set in a trough containing Schiff reagent and again refrigerated for 45 minutes. Destaining was accomplished in 2–3 rinses with 10% acetic acid at room temperature. *Isoenzymes* were determined using the staining procedures of Shaw (1965) with the following exceptions: peroxidase-Brewer's procedure (1970) was used; polyphenol oxidase—60 mg of catechol in 100 ml 0.01 M phosphate buffer, pH 6.8 was employed (Brown and Mazzone in preparation). The temperature of incubation for the staining procedures was 37°C except for esterase and peroxidase, which were incubated at room temperature.

Amino Acid Analysis. The blood of either insect was immediately freeze-dried and melanization did not occur. Blood was deproteinized with 1% picric acid

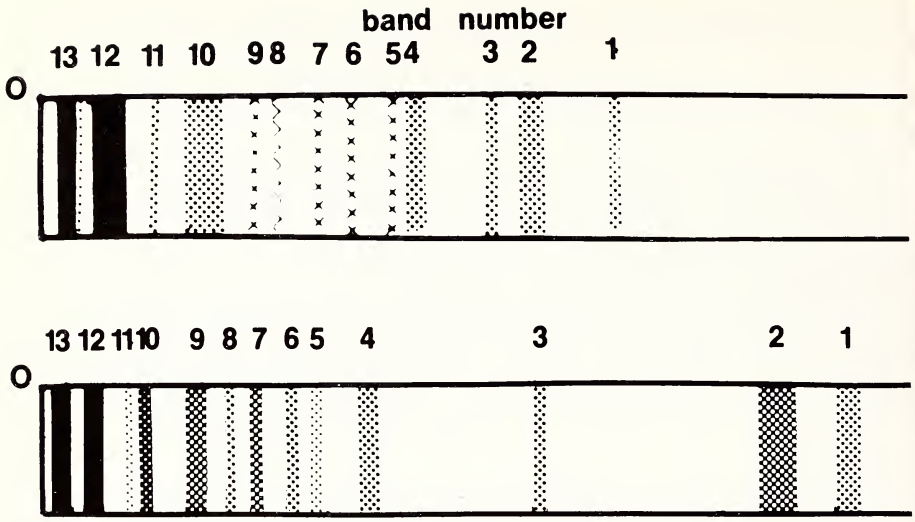

FIG. 1. Anodic polyacrylamide gel electrophoresis of hemolymph proteins. Top: 4th larval instar of the gypsy moth; Bottom: final larval instar of the European pine sawfly. O = origin. Refer to text.

and the supernatant passed through a Dowex 2-X8 resin (200–400 mesh, chloride form) to remove the picric acid. The effluent was evaporated to dryness and the residue analyzed with a Beckman Model 121 Automatic Analyzer (Stein and Moore, 1954; DeWolfe et al., 1967).

RESULTS

Color, pH, Visible Absorption Spectra, and Sedimentation Studies. Larval hemolymph of the gypsy moth had a blue-green color with an absorption maximum at 663 nm and a minimum at 520 nm. The pH of the blood was 6.57. Two sedimenting peaks were observed: a 17.6S major peak, which was 76% of the sedimenting material and a slower, 7.7S peak, representing 24% of the sedimenting material. Larval hemolymph of the European pine sawfly had a yellow color but did not exhibit a true spectral curve. Absorption decreased progressively from 400 nm to 800 nm. The pH of sawfly blood was 6.99 and one sedimenting peak with a value of 17.5S was observed.

Polyacrylamide Gel Electrophoresis. The protein patterns obtained are shown in Fig. 1. For each insect blood 13 bands were present, although occupying different sites along the gel. In each case, bands 13 and 12, the slowest migrating bands were predominant.

Lipids and Carbohydrates. In gypsy moth hemolymph lipids were detected in areas along the gel corresponding to protein bands 13 and 12, and in sawfly

Table 1. Isoenzymes in hemolymph of the gypsy moth (gm) and the European pine sawfly (Eps). The numbers refer to the corresponding protein bands as shown in Fig. 1. Italicized bands indicate areas of major enzymatic activity.

Isoenzyme	Corresponding Protein Band	
	gm	Eps
Adenylate Kinase	13,*12*,6,*4*,1	13,10,*3*
Esterase	13,*12*	13,*12*,10
Galactose-6-Phosphate Dehydrogenase	12,10,*7*,4,*2*	13,*10*,3
Fumarase	*13*,*12*,11,10,9–8,7,6,5,4,*3*,*2*,1	13,*10*,3
Hexokinase	13,12,10,8–6,5–4,*2*,1	*13*,10,5,4,*3*
Phosphoglucomutase	13,12,10,8–6,5–4,*2*,1	13,11,*10*,3
Xanthine Dehydrogenase	*13*,*12*,11–10,8–7,3,*1*	*13*,10,3
Peroxidase	12	13,12
Galactose Dehydrogenase	*13*,*12*,11,10,8,7,6,*4*,1	13,12,*10*,8,5,4
Polyphenol Oxidase	*13*,*12*,11	*13*,*12*,10
Malic Acid Dehydrogenase	12,*10*,9,8,2	*13*,*12*,10,*9*,4
Lactic Acid Dehydrogenase	*13*,*12*,11,10,9,7–6,*3*,*2*,1	13,*12*,*10*,8,7,5,*4*
alpha-Glycerophosphate Dehydrogenase	12,11,10,9,8,*7–5*,4,3,2,1	13,*12*,*10*,8,7,5,*4*
Alcohol Dehydrogenase	*12*,*11*,10–9,7–6,4,3,2,1	13,12,11,10,8,7,5,*4*
Creatine Kinase	*13*,*12*,9,8,7,6,5,4,*1*	
Glutamic Dehydrogenase	13,12,11,10,8–5,3,2,1	
Glucose-6-Phosphate Dehydrogenase	12,*10*,8–7,6–5,3,2	

hemolymph, bands 13 and 10. Carbohydrates were detected in areas along the gel in all bands for the gypsy moth except 6 and 1, and were present in bands 13, 12, 10, 8 and 3 of sawfly blood.

Isoenzymes. The isoenzymes present in the protein bands of each insect blood pattern are given in Table I. In gypsy moth blood 17 isoenzymes were observed, while in blood of the European pine sawfly, 14 isoenzymes were present.

Amino Acid Analysis. Table II presents the number and concentration of amino acids present. For gypsy moth blood, glutamine and lysine were present in the highest amount for the common amino acids. Tryptophane and hydroxyproline were absent. Urea, which was also measured in the amino acid analyses was present in relatively high concentration in the gypsy moth blood sample. In sawfly blood glutamine and asparagine were present in the highest amount for the more common amino acids, while sarcosine was present in the highest amount of all compounds measured. Aspartic acid was absent in sawfly blood.

DISCUSSION

The blue-green pigment of larval hemolymph of lepidoptera is believed to be a bile pigment (see Schmidt and Young, 1971) and possibly a mesobiliverdin (Van Der Geest, 1968). Larval hemolymph of the sawfly, although yellow in

TABLE 2. Amino acids and related compounds in hemolymph[a] of the gypsy moth (gm) and the European pine sawfly (Eps). a = μmoles of amino acid/compound in 10 mg of freeze dried blood; ND = not detected.

Amino Acid/Compound	gm	Eps	Amino Acid/Compound	gm	Eps
Tryptophane	ND	0.35	Aminoadipic Acid	ND	1.04
Lysine	2.88	1.28	Proline	1.44	1.44
Histidine	2.48	1.72	Glycine	0.44	2.12
Ammonia	0.40	0.84	Alanine	0.72	2.12
Arginine	0.80	0.49	Citrulline	ND	ND
Phosphoserine	0.04	0.08	Aminobutyric	ND	ND
Taurine	ND	0.16	Valine	0.72	0.28
Phosphoethanolamine	1.44	1.80	Half-Cystine	0.04	0.04
Urea	6.44	0.68	Cystathionine	0.56	0.20
Aspartic Acid	0.04	ND	Methionine	0.44	0.12
Hydroxyproline	ND	0.04	Isoleucine	1.84	0.24
Threonine	0.52	0.88	Leucine	0.36	0.20
Serine	0.72	1.44	Tyrosine	0.24	0.40
Asparagine	1.48	2.64	Phenylalanine	0.24	0.04
Glutamic Acid	0.17	0.11	B-Alanine	ND	0.92
Glutamine	4.80	3.90	Aminoisobutyric Acid	ND	ND
Sarcosine	ND	9.93			

color, did not exhibit a true spectral curve. Possibly there are interfering substances present which mask the true absorption of the prosthetic groups, which for yellow chromoproteins are believed to be B-carotene and lutein (Van Der Geest, 1968; Hackman, 1952). Studies on the characterization of the hemolymph pigments for these two insects will be reported in a subsequent study (H. M. Mazzone and S. E. Brown).

Heimpel (1961) found the pH of hemolymph of a number of insects to range from 6.2 to 7. For the gypsy moth a value of 6.75 for the last instar hemolymph was observed, compared to our value of 6.5 for hemolymph of the 4th instar. He observed a value of 6.81 for hemolymph of the last instar of the European pine sawfly compared to our value of 6.99 at the same instar.

A common sedimentation value of approximately 17S was observed for the fastest sedimenting peak in hemolymph of the gypsy moth and for the single component of the hemolymph of the European sawfly. Lauffer (1943) observed a main component in hemolymph of Bombyx mori having a value of 16S.

In the present study, protein electrophoretic patterns showed two predominant bands near the origin. The possibility of a "common" protein in insects has been suggested by Whittaker and West (1962) in their electrophoretic studies on 18 species of insects. In hemolymph protein patterns, which included a sample from the European pine sawfly, they observed a predominant band close to the origin in all cases. Hudson (1966) also referred to a "common" protein in hemolymph of *Protoparce quinquemaculata* and believed it to be

similar to a particular hemolymph protein in electrophoretic patterns obtained for *Hyalophora cecropia* and *Samia cynthia* (Laufer, 1960); *Malacosoma americanum* (Whittaker and West, 1962); and *M. americanum* and *Rotschildia orizaba* (Loughton and West, 1965). In these studies no sedimentation values were obtained for hemolymph proteins which might have supported the concept of common proteins in insects.

Lipoproteins and glycoproteins were observed in hemolymph of each insect reported in the present study. Indeed, carbohydrates were associated with all the protein bands of gypsy moth hemolymph, except bands 6 and 1. Bennett et al. (1968) reported lipids and carbohydrates in association with the main hemolymph protein of the japanese beetle, *Popilia japonica*. Siakatos (1960 a,b) suggested that the blood proteins serve as carriers of nutrients such as lipids and carbohydrates.

Creatine kinase, glutamic dehydrogenase and glucose-6-phosphate dehydrogenase were present in larval hemolymph of the gypsy moth but were absent in the European pine sawfly. A detailed study of isoenzymes in the metamorphic stages of the gypsy moth is under study (S. E. Brown and H. M. Mazzone). Isoenzymes in insects have been assigned various functions, e.g., PPO is involved in the tanning and hardening of the cuticle (Brunet, 1965) and in defense mechanisms (Taylor, 1969).

Amino acids in high concentrations of the gypsy moth were glutamine, lysine, histidine, isoleucine, and proline. Tryptophane, hydroxyproline, and sarcosine were absent. A detailed study of the amino acids in the metamorphic stages of this insect is in progress (R. T. Zerillo) and a high concentration of urea in hemolymph has been noted.

In hemolymph of the European pine sawfly sarcosine was present in the highest amount followed by glutamine, glycine, and alanine. Aspartic acid was absent.

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